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14. ABSTRACT
The aim of this Program is to study the association between epidemiologic risk factors, low-risk genes, and histologic and novel molecular subtypes of ovarian cancer. In December 2002, we received final approval from the Human Subject Research Review Board (HSRRB) to start recruitment at 13 of the proposed study sites and have since received approval to recruit at a further 2 sites. We have established a network of research nurses across the country and recruitment is now progressing well at 15 different sites. Recruitment of cases for the study began in January 2003 and control recruitment began in May 2003. Full population-based recruitment of cases ceased for women diagnosed after 30 June 2005 but collection of biospecimens and limited epidemiological data continued at key centres until June 2006. We have recruited a total of 1707 women with ovarian cancer (with an additional 296 women recruited since 1 July 2005 for the biospecimens extension) and 1073 control women. The recruitment, sample and data collection and processing systems have worked well and we are continuously monitoring our performance against our targets as outlined in the reports from Core Components A (Epidemiology) and B (Biospecimens).

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Molecular epidemiology, heterogeneity, histologic subtypes, ovarian cancer

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INTRODUCTION

The aim of this Program is to study the association between epidemiologic risk factors, low-risk genes, and histologic and novel molecular subtypes of ovarian cancer, thereby addressing the heterogeneity of the disease and of susceptibility to environmental exposures. To this end, we have established a multi-center population-based resource involving collection of linked epidemiologic and clinical data and biospecimens from cases and matched controls.

BODY

Cores A and B

In December 2002, we received final approval from the Human Subject Research Review Board (HSRRB) to start recruitment at 13 of the proposed study sites. During the final months of 2002 we identified Research Nurses for each study site and set up procedures at each hospital. By January 2003 research nurses were deployed at the collecting sites and we started phasing in recruitment. We obtained HSRRB approval to recruit at all 15 sites listed below. Full recruitment continued at all sites until June 30 2005 and recruitment continued at selected sites until June 30 2006. Participant recruitment is now complete and data/sample collection is will be complete within the coming weeks (see Reports for Cores A, Epidemiology, and B, Biospecimens, below).

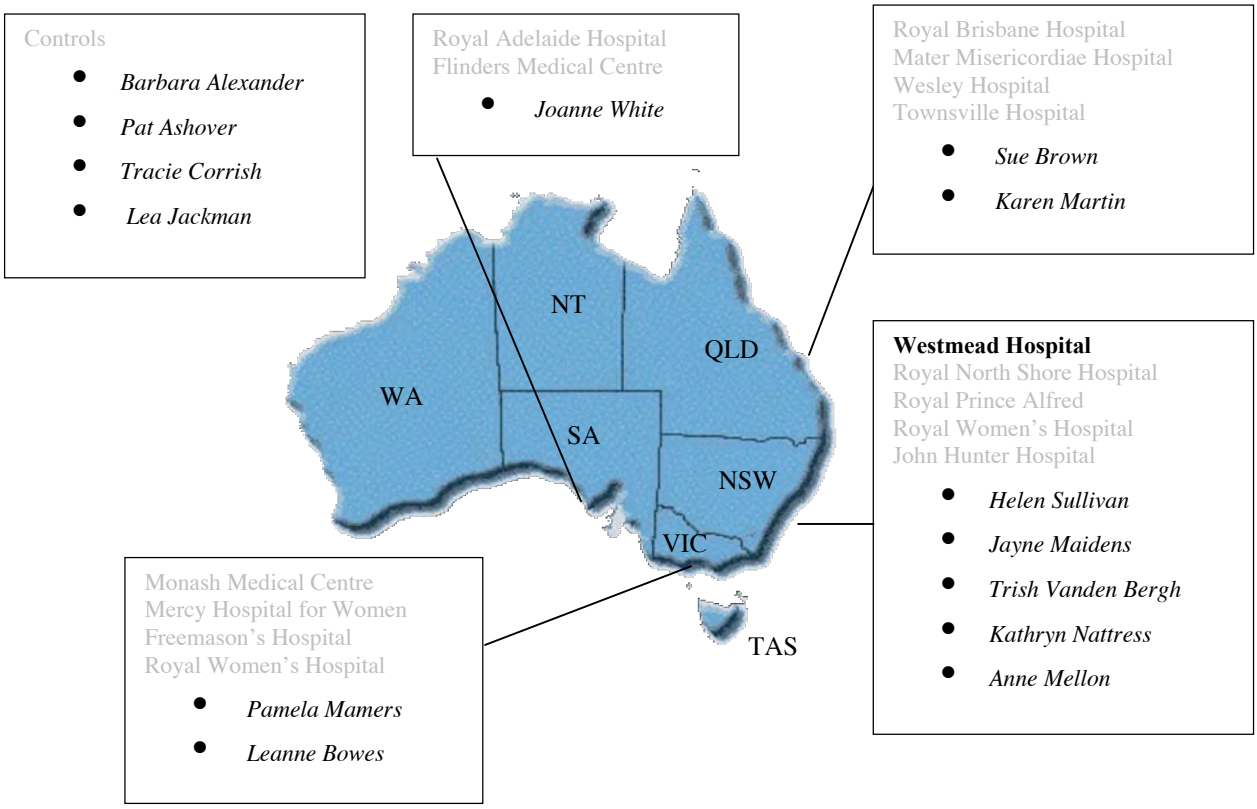

Figure 1: Australian Ovarian Cancer Study Site and RN network at 30 June 2006

Tasks Outlined in the Approved Statement of Work
Core A: Epidemiology

Task 1- Preliminary Work (Prior to start date)

(a) Data-collection instruments will be finalised and piloted based on practical experience in a previous study (Survey of Women's Health)

From 2002 Annual Report-

The **main study questionnaire** has been through an extensive development phase to ensure measurement of exposure to a comprehensive range of established and putative causal factors, potential confounders and novel factors of interest. Because the instrument was to be used by cases and disease-free controls from a range of educational backgrounds, we invested much effort in producing a document that was clear, simple and unambiguous. When the final content of the questionnaire was established, we focused on the style and formatting to ensure readability, clarity and consistency, and to ensure that items were relevant to people with and without disease. We then undertook pilot testing on a convenience sample of neighborhood controls from a range of educational and occupational backgrounds (specifically non-medical, non-research) to identify inappropriate use of jargon and potential sources of ambiguity or irrelevance. (Each pilot respondent was invited to provide direct feedback, and they did). The revised questionnaire was then tested in two groups of patients for whom clinical records were available to permit validation of responses. The returned questionnaires showed a high degree of completeness and the responses demonstrated a high level of internal and external validity.

The **food-frequency questionnaire** has been updated to reflect changing dietary practices since the original instrument was developed more than 10 years ago. We have also added additional items to address specific hypotheses concerning the role of soy products and xeno- and phytoestrogens in ovarian carcinogenesis. This questionnaire has been trialed in a group of cancer-free patients and minor modifications made to improve readability.

Case-recruitment: More extensive pilot testing of the study instruments and recruitment protocols has been conducted in Queensland using limited local funds. Women diagnosed with ovarian cancer at the Royal Women's Hospital, Brisbane (since 1 January, 2002) and at the Mater and Wesley Hospitals (since 1 July 2002) have been recruited and asked to complete the main study questionnaire. To date 102 cases have been identified of which 92 were potentially eligible and 82 (90% of eligible cases) have consented to participate. Tissue collection started in April 2002 and blood collection began in July and 34 tissue and 10 blood samples have been collected to date. We are in the process of cross-checking with the local Gynaecology-Oncology register and the State Cancer Registry to identify any cases we have missed and will then modify our ascertainment procedures if necessary to improve capture in the future.

Control recruitment: we are currently trialing the control recruitment methods in a separate study of oesophageal cancer. In response to the first batch of 49 study invitations sent, 32 men and women (65%) have agreed to participate to date, and a few people are still to be contacted. Participation rates are slightly higher among women than men thus we anticipate that we will be able to achieve the estimated participation rate of 70% for AOCS.

(b) Institutional Review Board approval will be obtained from all participating hospitals and institutions (to be prior to start date)

From 2003 Annual Report-

Institutional approval has been obtained from the following sites-

Queensland Institute of Medical Research	APPROVED 17/08/2001
Royal Women's Hospital, Brisbane	APPROVED 27/06/2001
Mater Misericordiae Hospital, Brisbane	APPROVED 23/10/2001
Wesley Hospital, Brisbane	APPROVED 19/06/2002
Peter MacCallum Cancer Institute	APPROVED 12/09/2001
Monash Medical Centre, Melbourne	APPROVED 10/05/2002
Mercy Hospital for Women, Melbourne	APPROVED 25/06/2002
Freemasons Hospital, Melbourne	APPROVED 14/03/2002
Royal Women's Hospital, Melbourne	APPROVED 29/02/2003
Westmead Hospital, Sydney	APPROVED 7/11/2001
Royal Hospital for Women, Sydney	APPROVED 11/10/2001
Royal North Shore Hospital, Sydney	APPROVED 12/04/2002
Royal Prince Alfred Hospital, Sydney	APPROVED 17/06/2002
John Hunter Hospital	SUBMITTED 31/10/2002
Royal Adelaide Hospital, Adelaide	APPROVED 22/01/2002
Flinders Medical Centre, Adelaide	APPROVED 21/02/2002

(c) **Identification of project manager, data manager and nurse-interviewers to start on Day 1.**

From 2003 Annual Report-

The Project Manager and Data Manager have been in place for 18 months and we now have a team of part-time nurse interviewers in place at hospitals across Australia:
Queensland (2): based at QIMR and covering all hospitals
New South Wales (4): based at RHW, RPA, RNS, Westmead (plus 1 RN ready to start at John Hunter when we receive approval)
Victoria (2): based at Monash and PMCC (covering Mercy/Freemasons).
South Australia (1): based at RAH

In many cases we have employed RN's on a part time basis who are also employed for other studies at the participating sites. This had a number of advantages: the RN's were known to the clinical staff and therefore readily accepted, they had an immediate understanding of clinical processes on site, and they could be employed for just the time that was needed. Employing part time RN's at each site, rather than one full-time person to cover several sites, avoided problems associated with travelling between sites during peak hour.

Task 2- Set-up (months 1-2)

(a) Finalise details of case identification system in each of the major centres (month 1)

From 2003 Annual Report-

The Epidemiology Project Manager (together with the Biospecimen Project Manager) has been working with clinicians, pathologists and nursing staff at each of the major centres to establish a system for case ascertainment and recruitment appropriate for each site. Processes for data and sample collection are also in place at each of the major centres. Once final approval is obtained, the research nurses will maintain regular contact with the

gynecology-oncology clinics and wards to identify eligible women as soon as possible after diagnosis.

We have participated in the Australian New Zealand Gynaecological Oncology Group (ANZGOG) meetings in 2001 and 2002, presenting details of the study on both occasions. ANZGOG is affiliated with the US GOG. There is comprehensive representation at the meetings by pathologists and gynaecological oncologists at the meetings and they have provided an excellent opportunity to provide information to key participants. There is enthusiastic endorsement of the study by ANZGOG members.

(b) Training of interviewers in Brisbane (month 1)

From 2003 Annual Report-

Once final approval is received, the Nurse Interviewers will be trained on-site by the Epidemiology and Biospecimen Project Managers and the specific recruitment details for each site will be finalised. We plan to bring all of the nurses to Brisbane in November for more detailed training and to trouble-shoot any problems that have arisen in the early stages of recruitment.

(c) **Development of computer data-bases (Access) for data-entry**

Recruitment "Tracking" Database

From 2003 Annual Report-

We have developed a secure internet-based database for the state-based study nurses to register newly identified cases and to allow us to track all stages of recruitment, consent and data and sample collection. The database is constructed using the MySQL Relational Database Management System and comprises 31 tables. A dedicated web server that sits behind the QIMR firewall provides the platform, with web services effected via the Apache web server that employs the Secure Socket Layer to provide an encrypted channel for all communication between client and server machines. The database has been pilot tested and is ready to come on line as soon as recruitment begins. The development of other linked databases for entry of questionnaire data is underway.

Main Questionnaire and Dietary Questionnaire Databases

From 2004 Annual Report-

We have developed and tested the database for the main study questionnaire, the dietary questionnaire and the interview and data entry is on-going (see below). The databases incorporate multiple range and logic checks to prevent errors in data entry.

Task 3- Recruitment of cases (n>1000). (Ongoing months 2-36)

(a) Cases will be identified by the nurse-interviewers on an ongoing basis through participating hospitals and clinics with additional checks run through the state cancer registries

(b) Treating physicians will be contacted to obtain permission to contact the case

(c) Cases will be contacted and interviewed and biological samples collected

(d) Tumor blocks and copies of pathology records will be obtained

Population-based recruitment was phased in from 1 January 2003 and continued to include women diagnosed up to June 30 2005. Although, for financial reasons, the recruitment period was 5 months shorter than initially planned, we exceeded our target of

more than 1000 women and more than 600 fresh tissue samples (Table 1). Final collection of outstanding questionnaires and biospecimens is complete.

Case ascertainment: The total numbers of eligible cases in each state are estimated from numbers of ovarian cancers registered in each state in 2001 (the most recent data available). We were aiming to ascertain 100% of cases in Qld and SA and 50% in Victoria and New South Wales and have met or exceeded these targets in all states except Victoria where recruitment at one major hospital did not start until 1 Jan 2004 (because of overlap with an existing study at that site).

Response rate: We were aiming for a response rate of 85-90% and a total case group of 1000. Overall 88% of eligible cases who were approached about the study (76% of those identified) consented to take part, a total of 1707 women (NB this includes 186 women ascertained prior to Jan 2003 during a pilot study in Queensland that was funded separately). Case ascertainment therefore exceeded initial projections by close to 50%. Overall, 86% of participating women completed a questionnaire and we collected blood and tissue samples for 85% and 52% respectively. After pathology review, 1165 women (69%) were confirmed as having primary epithelial cancer of the ovary, peritoneum or fallopian tube, of whom 1092 returned a questionnaire, 1022 gave a blood sample and we obtained a fresh tissue sample for 600 and so were eligible for inclusion in analyses. We therefore met or exceeded all of our recruitment targets.

Table 1: Full Population-Based Case Recruitment: Women diagnosed to 30 June 2005*

	NSW	VIC	QLD	SA	TOTAL
Total cases (estimated)[1]	**850**	**830**	**707**	**213**	**2600**
Cases ascertained	**601**	**368**	**994[2]**	**296**	**2259**
% of total (see Fig 2)	71%	44%	141%	139%	87%
Cases included[2]	**509**	**338**	**861**	**240**	**1948**
% of those ascertained	85%	92%	87%	81%	86%
Consents	**445**	**311**	**750**	**201**	**1707**
% of included	87%	92%	87%	84%	88%
Q returned to QIMR	**373**	**255**	**668**	**169**	**1465**
% of consents	84%	82%	89%	84%	86%
Fresh tissues	**259**	**198**	**292**	**102**	**851**
% of consents	58%	64%	39%	51%	52%
Blood samples	**358**	**290**	**649**	**156**	**1453**
% of consents	80%	93%	87%	78%	85%
Urine samples	**284**	**133**	**261**	**0**	**678**
% of consents	64%	43%	35%	0%	40%

[1] Based on number of cases diagnosed in 2001 (most recent data available). We only aimed to ascertain 50% of cases in NSW and Vic

[2] Includes 255 women ascertained during a pilot study in 2002 (funded separately). Women were excluded if they were unable to give informed consent (eg non-English speaking, psychological reasons, too sick)

We faced a funding shortfall amounting to 5 months of collection due to US-Australian exchange rate variation. However, given our success during the planned case ascertainment period, additional funding was obtained to a) allow collection for the full

period initially specified (36 months) plus b) an additional 6 months (to June 2006). This additional recruitment is now complete and data/sample collection is being finalised.

Table 2 shows case recruitment and sample collection from July 1 2005 to 30 June 2006.

Overall, 2244 cases were recruited and of these 1965 represented eligible cases (LMP or primary epithelial invasive ovarian cancer), with the majority of the remainder representing benign or secondary ovarian neoplasms. From these, a total of 1061 fresh frozen tumour samples were collected (Table 3).

Table 2: Supplementary Clinic Based Case Recruitment: 30 June 2005 to 30 June 2006

	NSW	QLD	SA	VIC	TOTAL
Cases ascertained	**78**	**99**	**87**	**38**	**302**
Cases included	**76**	**99**	**83**	**38**	**296**
% of those ascertained	97%	100%	95%	100%	98%
Consents	**73**	**76**	**72**	**37**	**258**
% of included	96%	77%	87%	97%	87%
Q returned	**71**	**76**	**71**	**36**	**254**
% of consents	97%	100%	99%	97%	98%
Fresh tissues	**70**	**46**	**65**	**29**	**210**
% of consents	96%	61%	90%	78%	81%
Blood samples	**66**	**71**	**71**	**33**	**241**
% of consents	90%	93%	99%	89%	93%

Table 3: Total number of included AOCS cases recruited to 30 June 2006

	NSW	QLD	SA	VIC	TOTAL
Cases ascertained	**679**	**1093**	**383**	**406**	**2561**
Cases included	**585**	**960**	**323**	**376**	**2244**
% of those ascertained	86%	88%	84%	93%	88%
Consents	**518**	**826**	**273**	**348**	**1965**
% of included	96%	77%	87%	97%	87%
Q returned	**444**	**744**	**240**	**291**	**1719**
% of consents	86%	90%	88%	84%	87%
Fresh tissues	**329**	**338**	**167**	**227**	**1061**
% of consents	64%	41%	61%	65%	54%
Blood samples	**424**	**720**	**227**	**323**	**1694**
% of consents	82%	87%	83%	93%	86%

Task 4- Recruitment of population controls (n>1000). (Ongoing months 2-36)

(a) Potential controls will be selected at random through the Commonwealth electoral roll on a weekly basis and frequency matched by age and geographic region to the distribution of cases identified the previous week
(b) Invitation letters will be sent to controls
(c) Telephone follow-up of controls
(d) Interview of controls and collection of blood and urine samples

Control recruitment is complete with a total of 1073 women consenting to take part. We have therefore exceeded our target of 1000 control women. Of these women 1072 (99.9%) returned a completed questionnaire and 924 (86%) provided a blood sample. We found that, as for other population-based studies, it is getting harder to persuade disease-free people to take part and our final completion rate was lower than expected with only 47% of those contacted agreeing to participate. To ensure that this will not introduce unacceptable bias into our epidemiological analyses in Project 2 we have compared key characteristics of our control group to national Australian data. Our control group appears to be very representative of the population in terms of education level, parity and body size and while there are minor differences for some other variables (for example current smokers are slightly under-represented and oral contraceptive users are slightly over-represented) we will conduct sensitivity analyses to estimate the potential effects of any bias and are confident that this will not unduly affect our results. Furthermore, the majority of analyses will compare risk factors for the different histological subtypes of ovarian cancer and these case-case comparisons will not be affected. The low response rate is also very unlikely to affect the genetic analyses in project 3.

Task 5- Data entry / checking / cleaning. (ongoing months 3-42)
(a) Data will be entered into the databases on an ongoing basis
(b) Data will be cleaned using frequency and range checks, implausible values will be cross-checked against the original questionnaires and corrected if necessary

Data entry for the full population-based study is complete and the core variables have been cleaned for analysis (see project 2).

Task 6- Data management (ongoing months 37-48)

We have derived the key variables for analyses and these are reported under the individual projects.

<u>Core B- Biospecimens</u>

Task 1- Preliminary Work (Prior to start date)

a) Institutional Review Board approval will be obtained from all participating hospitals and institutions (to be prior to start date)

From 2003 Annual Report-

Institutional Review board approval has been obtained from all participating hospitals and institutions- see summary above.

b) Recruitment of data manager and specimen processing staff

From 2002 and 2003 Annual Reports-
A Project Manager (Nadia Traficante) has been recruited to coordinate the biospecimen core and oversee management of specimen processing staff. To date, this has involved establishing biospecimen collection and processing protocols, ordering consumables and liasing with clinicians, pathologists and ward staff to establish site-specific sample collection procedures.
A Data Manager (Sian Fereday) has been recruited to develop the biospecimen core databases and manage data entry staff.
In addition, Joy Hendley and Lisa diPrinzio (biospecimen processing staff) have been recruited to process blood, tissue and urine samples and to complete data entry for the biospecimen core.

c) **Further refinement of computer data-bases (Access) for data-entry**
From 2002 Annual Report-
Further refinement of the biospecimen database to ensure the appropriate tracking from collection through processing to final usage of all specimens has been completed. In addition, the database also allows all biospecimen data to be linked to diagnostic pathology data and central pathology review information.

Task 2- Set-up (months 1-2)

a) Finalise details of case ascertainment system in each of the major centres (month 1)
From 2002 Annual Report-
The Project Managers have been working with clinicians, pathologists and nursing staff at each of the major centres and established a system for case ascertainment and recruitment at each of the collaborating sites. Processes for data and sample collection are also in place at each of the major centres. Once final approval is obtained, the research nurses will maintain regular contact with the gynecology-oncology clinics and wards to identify eligible women as soon as possible after diagnosis.

b) Obtain minor equipment and consumables
From 2002 Annual Report-
We have purchased a Liquid Nitrogen tank for storage of blood, tissue and urine samples. Minor equipment (gilsons, pipettes) and consumables (including blood, tissue and urine collection tubes, syringes, needles, scalpel blades, forceps, reagents for specimen processing, Guthrie cards, gloves, tips, bio-bottles and consignment notes) for the biospecimen core have been purchased.

Task 3- Ascertainment of samples. (Ongoing months 2-36)

a) Nurse-interviewers to liase with Biorepository head, notifying of incoming shipment of samples
b) Nurse-interviewers to provide Biorepository staff with details of pathology blocks from cases to be requested. Biorepository staff to coordinate temporary block acquisition
c) Blood and urine samples shipped overnight from national sites. Fresh frozen samples from interstate stored at centres at –80°C, shipped on dry ice at monthly intervals. Blocks from pathology clinics requested on a monthly basis.

Sample collection for the biospecimen core was phased in from 1 January 2003 and

continued to include women diagnosed up to June 30 2005. Further recruitment and collection of biospecimens and limited epidemiological data continued at major centres to include women diagnosed up to June 30 2006 under an extension to the original grant. This additional recruitment is now complete and data/sample collection is being finalised. Table 3 above shows the total number of tissue and blood samples collected from January 1 2003 through to June 30 2006.

Table 4 below shows the total number of primary epithelial ovarian, peritoneal and fallopian frozen tissue samples collected (LMP and Invasive). It is noteworthy that the heterogeneity of ovarian cancer resulted in extensive stratification of sample types. By extending sample collection, we were able to collect 630 serous invasive cancers, as well as less common subtypes, including 58 invasive clear cell and 90 invasive endometriod cancers. The number of primary invasive mucinous ovarian cancers is lower than in some reports, however, this reflects the increasing recognition that invasive mucinous tumours of the ovary are usually secondary neoplasms (*Lee et al*).

Table 4: Total number of AOCS frozen tissue samples by subtype*

Type	Primary Site	Total	Clear Cell	Endo	MMMT	Muc	Other	Ser/PapSer
LMP	Ovary	200		2	1	101	23	73
LMP	Peritoneum	3				3		
Malignant	Fallopian	15		2			1	12
Malignant	Ovary	742	56	89	23	31	60	483
Malignant	Peritoneum	147	2	1	1	1	7	135
Total		**1107**						

** the total number of frozen tissue samples collected is higher than that reported in Table 3 above, as this figure includes cases recruited from Western Australia and Tasmania with local funding (these states were not part of the original DoD grant)*

**other includes Brenner tumors, tumors of mixed subtype and poorly differentiated carcinomas where subtyping is not confirmed.*

Blood samples were shipped at room temperature on a daily basis to the Biorepository and nurse-interviewers liased directly with the Biorepository head, notifying of incoming samples. This was usually via email. Fresh frozen tissue samples and urine samples were shipped on dry ice on a monthly basis via overnight courier. Again, the nurse-interviewers notified the Biorepository head of all incoming samples.

Nurse-interviewers collected both fresh frozen and fixed tissue samples. The fixed samples shipped to PMCC on a daily basis were processed into blocks.

In addition, an expert gynaecological pathology review panel was established to review all cases recruited to the study. The process is ongoing and involves diagnostic slides and blocks being called in from pathology centres (where the case was first diagnosed) for centralized review. This protocol has been in place for several years to facilitate ovarian cancer research in Australia and is being co-ordinated by Dr Peter Russell and the Biorepository head.

Task 4. Sample processing and dispatch. (Ongoing months 2-36)

a) Incoming samples of blood, urine, fresh frozen tissue and blocks to be processed as described in methods

b) Requested samples shipped to centres in general on a monthly basis but immediately available if needed
c) Sample backup to QIMR sent as batches on a monthly basis
d) Periodic quality control procedures to validate sample integrity

Joy Hendley and Lisa DiPrinzio were responsible for all biospecimen sample processing. Incoming blood, urine and tissue (frozen and fixed/blocks) samples were processed according to the AOCS protocols. A backup sample is stored separately (liquid nitrogen and -80°C) and was sent via overnight courier to QIMR on a monthly basis.
We established quality control protocols and the biorepository staff were responsible for their implementation.

Task 5. Data entry / checking / cleaning. (ongoing months 3-42)

a) Data will be entered into the databases on an ongoing basis
b) Provide data for analysis as required

Biorepository staff were responsible for entering information regarding sample collection (type of sample, date collected, date processed) and processing (fractions processed, amount processed, storage location) onto the biospecimen database.

The Data Manager, Sian Fereday, is responsible for generating monthly statistics for the management group meetings. These reports describe primary site, histology subtype and stage of the biospecimens collected.

Adhoc data requests are furnished as required.

Project 1: Molecular Subtype Analysis of Ovarian Cancer

Task 1. Initial DNA microarray analysis with ~300 archival fresh frozen samples (months 1-12)

From 2004 Annual Report-

Utilising retrospectively collected specimens of ovarian cancer (OvCa) a database of gene expression information has been created. The data were generated using custom printed cDNA microarray slides from The Peter MacCallum Cancer Centre Microarray Facility. These arrays contain approximately 10,500 genetic elements and have been used to profile differences in gene expression between various clinically important classes of OvCa. Over 100 samples have been profiled to date and the data analysed to investigate three major questions: correlation of expression profile with outcome, classification of Low Malignant Potential (LMP) tumours, and classification of Krukenberg tumours.

Outcome: Genes with robustly different patterns of expression between patients with short or long survival times may be important targets for novel diagnostic or therapeutic tools. To explore this potential, analysis of array data generated from patients following standard treatment regimes was conducted. Groups of molecular profiles from patients with survival times <12 months, 12-24 months and >24 months were created. Pattern recognition algorithms were used to identify genes from the 10,500 available, whose expression correlated significantly with the survival phenotype. From this analysis a

prediction model was created which was able to correctly assign patients to one of either three survival categories with approximately 80-90% accuracy. In addition, a linear formula was generated, which was capable of predicting survival time as a continuous variable from gene expression data. Importantly genes identified by the algorithms used in this study included several genes of known importance in OvCa prognosis, including TYROBP, CXCL9, CCL8 and MT1G as well as several other genes not previously shown to correlate with patient outcome. Kaplan Meier analysis of the predictions made from this analysis revealed a highly statistically significant difference between survival classes. Further investigation and validation of these findings is planned using additional specimens from Westmead hospital (see Task 2 below)

LMP tumours: LMP tumours are an unusual class of ovarian tumours that display a relatively indolent pattern of disease, despite frequently having mutations in the ras-MAPK pathway. We have used microarrays to explore global gene expression patterns differences in mucinous invasive, mucinous LMP, serous invasive and serous LMP tumours. A multivariate method of microarray data analysis has been conducted whereby variation in expression attributable to histology, for example, is controlled for thus revealing genes with expression patterns influenced by or responsible for the phenotypes of interest. Gene ontology, genomic and pathway information was used to elucidate the molecular processes that differentiate LMP from invasive tumours and we have compared these with similar datasets for other solid cancers, particularly breast cancer. In addition, we are comparing expression patterns of LMP tumours for which we have determined their k-ras and BRAF mutation status.

We have identified a set of genes with robust expression differences between LMP and invasive tumours. This subset contains a significant representation of antigen presentation, cell-cycle regulation, control of cell growth and adhesion genes. We have shown that the LMP/invasive expression signature is similar to that observed in published expression analyses of breast ductal carcinoma in-situ versus invasive ductal carcinoma, and low versus high-grade breast cancer.

A highly significant proportion of the genes that discriminate between LMP and invasive ovarian cancer were located on chromosome six ($P<0.001$). In particular, large differences in expression of genes at previously published areas of LOH on chromosome 6. Gene expression in these areas, known to contain tumour suppressor and apoptosis regulating genes, appears to be strongly inversely correlated between invasive and LMP tumours, with areas of under expression in invasive tumours associated with previously defined regions of LOH and gain, with regions of amplification. These findings suggest that copy number and gene expression changes on chromosome 6 may fundamental differences in growth and invasion between invasive and LMP tumours.

Krukenberg tumours: Krukenberg tumours are gastric tumours that metastasize to the ovary but other sites, including pancreas, breast and colon are common sites of origin for Krukenberg-like tumours. Whilst patients with these tumours may be recognized at surgery or upon pathological assessment, the clinico-pathological picture is often uncertain or such patients may simply go unnoticed. Treatment of such cases with a platinum-based regimen is usually ineffective. Our findings indicate that expression profiling allows the rapid identification of unrecognized metastases to the ovary and may be of use in the clinical management of the disease.

We have also developed a machine learning-based test to more accurately diagnose ovarian cancers. Initially, a cross-validated model of gene expression in primary ovarian cancer vs. over 100 other primary tumours was created and applied to LMP/invasive tumours to ensure the dataset is not contaminated by metastases from other tissues. AOCS has provided a very large, population-based, series in which we can estimate frequencies of primary and secondary mucinous cancers of the ovary using newer pathological criteria and our molecular classification. We have found that a higher fraction of invasive mucinous ovarian tumours represent secondaries, rather than primary tumours, than generally appreciated. We believe that the classifier may be used in conjunction with other clinical parameters to facilitate the diagnosis of mucinous invasive tumours of the ovary.

Task 2. Progressively switch to microarray analysis of prospectively collected samples (months 12-42)

The major focus of this project continues to be the analysis global gene expression of ~500 tumours using Affymetrix U133$^{2.0+}$ arrays. Gene expression profiling of prospective samples is progressing as planned. Three hundred and ten (310) samples have been gene expression profiled. Compared to published data, this represents the largest number of ovarian cancer samples analysed on complex arrays to date. The cases represent multiple histologies, stages, grades and clinical outcomes allowing for multiple questions of clinical and biological importance to be addressed. For example, 193 serous papillary cases with complete stage, grade and treatment records have been profiled and are suitable for analysis of prediction of outcome following chemotherapy.

Initial analysis of the expression data indicates that sample quality and array data is consistently excellent. Samples are frozen sectioned and reviewed for normal tissue, percent epithelial component, presence of necrotic tissue and consistency with the original pathology diagnosis. Additional sections are taken and are processed if the pathology review suggests that they are acceptable, that is they contain a high proportion of viable tumour with little normal tissue contamination (eg omentum or ovary). Samples are processed using Trizol and RNeasy and RNA quality accessed using a BioAnalyser, degradometer software, and a NanoDrop for quantitation. We are still following the strict quality control (QC) standards for RNA processing and quality suggested by Affymetrix and by the Hammersmith Microarray Core facility. RNA extracted from the fresh-frozen tissue collected for this study has consistently been of high quality, with no more than 2-3 cases having RNA unsuitable to proceed with gene expression profiling

Once hybridized, a comprehensive assessment of array quality is performed. This includes a review of the Affymetrix Gene Chip Operating Software reports produced after each array has been scanned. The scaling factor, percent present genes, the 3' to 5' ratio (indicating labelling efficiency)**,** the background, and the spike-in controls (indicating hybridization efficacy) are always reviewed to ensure that every array meets the specified standards. To date, only 3 arrays have been eliminated from subsequent analysis as a result of problems with these preliminary QC measures. A more comprehensive assessment using graphical tools provided in BioConductor has facilitated the decision of whether to remove an array from further analysis. Large batches of randomly selected arrays (25 -30 at a time) are compared to one another to identify outliers. Summary plots such as histograms and boxplots, and more advanced procedures, such as fitting probe level models and summarizing residuals and weights from these fits,

have been applied to all arrays generated to date. No additional arrays have been eliminated from subsequent analyses, indicating that the methodology used in generating the arrays has been of high standard, and has remained consistent for the duration of the study thus far.

Dr Anna Tinker, in collaboration with Dr.Adam Kowalczyk (Principal Researcher with Statistical Machine Learning, NICTA, Canberra, Australia) and his post-doctoral fellow, Dr. Brian Parker, have commenced the process of data analysis (details discussed below, Task 3).

Combined DNA copy number and gene expression analysis in serous ovarian cancer

We have performed a study of DNA copy number change (CNC) using 58 cases of Stage III/IV serous ovarian tumours using Affymnetrix 50K SNP arrays and 12 normal DNA controls from healthy donors. Assay performance has been consistently high, as indicated by signal detection levels greater than 99% across all experiments. High SNP call rates (>90%) were also observed in both the normal DNA reference and tumour samples.

In collaboration with the Broad Institute of MIT and Harvard (Boston) we have performed extensive analysis of CNC across all tumours. Both frequency and level of SNP copy number (low level to high level amplifications, single and homozygous deletions) were considered when mapping the most significant regions of change. We have found that as the sample size increases we are able to more precisely map minimal regions of CNC and find stronger associations with clinical endpoints.

14 highly-significant regions of amplification and 18 region of deletion have been identified and include novel and previously reported amplifications at 8q24.21 (containing MYC) and 3q26.2 (containing PKCi). We are currently further defining and characterising the remaining regions and correlating this to the larger expression data set. The combined analysis will enable us to specifically identify candidate oncogenes and tumour suppressors within regions of CNC.

We have also begun to identify correlations between individual regions of CNC and clinical parameters including platinum resistance, progression-free survival and over-all survival. These regions and the genes within will be the focus of further validation studies. We are also looking for linked regions of CNC to define tumour molecular subtypes and whether any of these are more specifically associated with clinical outcomes of interest.

Contiguous point analysis of SNP genotypes has also been applied to predict regions of LOH without reference to a matched normal sample. LOH events are frequent, however we have also identified a subset of tumours showing low levels of LOH. Despite the care taken in selecting samples of high tumour content and needle-dissection of samples, it appears that low levels of LOH detection may be due to remaining contamination from surrounding normal cells. As LOH analysis is particularly sensitive to contamination (as opposed to CNC which is more robust) we believe it is necessary re-run these samples after lase-capture microdissection. This will be performed on sections immunohistochemically stained to highlight surrounding fibroblasts and inflammatory cells for increased precision in isolating tumour cells.

Analysis of BRCA1 and BRCA2 tumours

Approximately 10% of cases in population-based series carry germline mutations in BRCA1/2 and therefore that we can expect some of the AOCS cases, especially those with serous ovarian cancer, to be from mutation positive women. If there is an expression signature in tumours arising in such women, it may possible to identify women who might benefit from mutation testing, however, Jazaeri et al (JNCI 2002, 94: 990-1000) reported that mutation negative tumours resembled either BRCA1 or BRCA2 mutation positive tumours. We would like to recapitulate the Jazaeri et al study and have begun to accumulate tumours from carriers. To date, we have ovarian tumours from 20 BRCA1 carriers and 11 BRCA2 and are seeking further samples for analysis.

Task 3. Ongoing statistical analysis of expression results (months 3-42)

Data will be hierarchically clustered to explore naturally appearing relationships, particularly when exploring samples with apparently similar clinico-pathological features such as an analysis of invasive serous cancers to search for unique molecular subsets.

Supervised data analysis can be used for other work where clinical or histological information separates groups, such as a comparison of LMP and invasive cancer or relating expression profiles to treatment response or survival. Currently there is no single 'best method' for associative analysis of genomic and clinical data and it is an area that is still under active development. We have a number of commercial software packages for microarray analysis and public domain tools that allow supervised analysis. In addition, we are working collaboratively with Dr Terry Speed's bioinformatics group, based at Walter and Eliza Hall Institute and with Dr Adam Kowalczyk's Statistical Machine Learning group, based at the National ICT Centre of Excellence (NICTA) in Canberra.

Genes will be tested using existing methods of feature-by feature statistical analysis including t-test, signal: noise metric, ANOVA and the score statistic (for Cox regression), with appropriate corrections for multiple testing using methods, in particular q-values, as well as empirical machine learning approaches. These methods are suited to an analysis of response that is considered as a categorical variable (resistant, sensitive). We will also treat outcome as a continuous variable, where disease free survival is used directly in the estimation process. Predictive signatures of resistance will be tested using Kaplan-Meier analysis.

In analysing the data we will explore whether the analysis should be done with cases that represent clinical extremes, such as progression on primary treatment versus long-term survival, or whether the dataset can be analysed unselected. The former has the advantage that by choosing biological extremes, key genes may be more readily apparent. The disadvantage of this approach is that it relies on analysis of a smaller subset of samples than if the data was analysed without prior selection (beyond the initial classification of resistant and sensitive samples). In all analyses samples will be withheld by splitting the microarray data into multiple permutations of training and independent sets, and using leave one out cross validation. These methods should identify genes that are significantly correlated with outcome. Over expressed genes and regions of amplification or loss will be investigated using human reference genome assemblies such as Golden Path Genome Browser (University of California Santa Cruz) or Ensembl

(EMBL - EBI and the Sanger Institute) to identify candidate oncogenes and tumour suppressor genes. To assess possible involvement of selected genes in related processes we determine whether specific GO annotations are enriched (http://www.geneontology.org/; http://david.niaid.nih.gov/david/ ease.htm) and use other commercial and public domain approaches to text and data mining to associate genes with pathways, such as Pathway Assist (http://www.stratagene.com/) and Ingenuity Pathway Analysis (http://www.ingenuity.com/). Techniques such as Gene Recommender will be used to identify genes that are correlated with members of the predictive list. Data will be compared with large meta-analyses of different solid tumours to determine whether there are relationships between found genes and other data sets. We will test whether genes that are predictive of treatment response are correlated with expression patterns of response and survival for other tumours, such as lung (NSCLC), bladder, oesophageal cancer, where platinum is a mainstay of treatment. Where possible, we will determine whether our gene lists are associated with histological, clinical or biological parameters in other ovarian cancer datasets.

Task 4. Full statistical analysis of expression data and preparation of manuscripts (months 42-48)

See above

Project 2: Determinants of Epithelial Ovarian Cancer- by histologic subtype and tumor behaviour

Task 1: Final preparation of data (month 1):

(a) Final checking of dataset to eliminate outliers

(b) Categorization of continuous variables (eg years of OC use) and generation of key derived variables (eg number of ovulations, body-mass index) to create standard variables for analyses

Completed

Task 2: Testing of hypotheses relating to reproductive factors:

(a) Analysis by histologic subtype

(b) Analysis by tumor behavior (low malignant potential vs invasive)

(c) Combination of (a) and (b) as required depending on previous results

(d) Combining of new data with existing data from SWH

We have completed analyses comparing reproductive risk factors for primary invasive serous cancers of the ovary peritoneum and fallopian tube. These represent the first case-control comparisons for primary peritoneal and fallopian tube cancers and have shown that while risk factors for fallopian tube cancer closely parallel those for ovarian cancer, there are some striking differences between primary ovarian and peritoneal cancers. These results have implications for our understanding of the etiology of ovarian cancer and we have submitted a manuscript to JNCI (see task 6).

Further analyses comparing risk factors for benign, borderline and invasive serous tumors of (a) serous and (b) mucinous histology are underway and final manuscripts will be submitted for publication within the next few months.

Task 3: Testing of hypotheses relating to exogenous factors (cigarette smoking, alcohol etc)**:**

(a) Analysis by histologic subtype
(b) Analysis by tumor behavior (low malignant potential vs invasive)
(c) Combination of (a) and (b) if required
(a) Combining of new data with existing data from SWH

We have begun analyses of the relation between alcohol consumption, different types of alcohol and patterns of alcohol consumption and aim to have a manuscript for publication by early 2007.

Task 4: Testing of hypotheses relating to health-related factors (endometriosis, obesity etc)**:**

(b) Analysis by histologic subtype
(c) Analysis by tumor behavior (low malignant potential vs invasive)
(d) Combination of (a) and (b) if required
(e) Combining of new data with existing data from SWH

We have begun analyses of the relation between endometriosis and body-size and risk of ovarian cancer, by histologic subtype, and aim to have manuscripts for publication by the end of 2006.

Task 5: Testing of hypotheses relating to androgen exposure (polycystic ovary syndrome, hirsutism, acne etc)**:**

(a) Analysis by histologic subtype
(b) Analysis by tumor behavior (low malignant potential vs invasive)
(c) Combination of (a) and (b) if required
(d) Combining of new data with existing data from SWH

Ongoing

Task 6: Preparation of manuscripts for publication

Several publications are currently in preparation. See Reportable Outcomes below.

<u>Project 3: Low-risk genes for epithelial ovarian cancer</u>

Task 1. To establish the 16 single nucleotide polymorphism (SNP) genotyping assays, including identification of genotyping controls (months 1-18)

Task 2 To genotype the cases from the Survey of Women's Health Study and controls from the Australian Breast Cancer Family Study for 16 SNPS (months 6-24)

Genotyping has been completed for ~550 ovarian cancer cases and 300 healthy controls for 11 SNPs as indicated in the table below. Four SNPs have been excluded from genotyping because of their low frequency (0-0.5%) detected in a sample of 90-125 Australian controls [published frequencies: 4% for *HSD17B1*:A-27C (Peltoketo et al, 1994); 2.4% for *RAD50*:Arg884His (http://greengenes/llnl.gov/dpublic/ secure/reseq) and 2% for *RAD52*:Ser347Ter (Han et al, 2002)].

Gene	Polymorphism	Status

Androgen Receptor (*AR*)	CAG_n	Spurdle et al., 2002
Progesterone Receptor (*PR*)	C44T	Not Commenced
Progesterone Receptor (*PR*)	G331A	Berchuck et al., 2004
Aromatase (*CYP19*)	C>T 3'UTR	Completed
5alpha-reductase (*SRD5A2*)	Val89Leu	Completed
17βhydroxysteroid dehydrogenase (*HSD17B1*)	A-27C	Excluded
17βhydroxysteroid dehydrogenase (*HSD17B1*)	Ala238Val	Completed
17βhydroxysteroid dehydrogenase (*HSD17B1*)	Ser313Gly	Completed
17βhydroxysteroid dehydrogenase (*HSD17B4*)	Trp511Arg	Completed
BRCA2	Asn372His	Auranen et al., 2003
X-ray cross complementation (*XRCC2*)	Arg188His	Webb et al., 2004
X-ray cross complementation (*XRCC3*)	Thr241Met	Webb et al., 2004
X-ray cross complementation (*XRCC3*)	CA_n	Not Commenced
RAD50	Arg884His	Excluded
RAD52	Ser347Ter	Excluded
RAD52	Tyr418Ter	Keleman et al, 2004

Analysis of the ***RAD52*** Y415Ter, ***XRCC2*** R188H G>A and ***XRCC3*** T241M C>T polymorphisms revealed no difference in genotype distribution between cases and controls. There was no increased risk of cancer associated with heterozygous genotype of *RAD52* Y415Ter (OR 0.55; 95% CI 0.24-1.24) (Keleman et al., 2004); *XRCC2* GA/AA genotype (OR 0.77; 95% CI 0.51-1.14), or with the *XRCC3* CT or TT genotypes (OR 0.80; 95% CI 0.59-1.09 and OR 0.92; 95% CI 0.58-1.44, respectively) (Webb et al., 2004). There was also no indication that genotype frequency differed across ovarian cancer subgroups defined by tumour characteristics, including histology. P53 positive tumours seemed to be over-represented in carriers of the *RAD52* truncation polymorphism (100% of 7 carriers but only 64% of 142 non-carriers were p53 positive). Although the rarity of the variant genotype frequency provided little power to detect modest risks in cancer for the *RAD52, XRCC2* and *XRCC3* variants, the data suggest that none of these variants play a major role in predisposition to ovarian cancer risk at the population level.

In contrast, in collaboration with Drs Easton et al in Cambridge, we have found an association between the Asn372His genotype of ***BRCA2*** and ovarian cancer risk (Auranen et al., 2003). We genotyped a total sample of 1121 ovarian cancer cases and 2643 controls. There was no difference in genotype frequency between control groups from the two Australian and British studies (P=0.9). The *HH* genotype was associated with an increased risk of ovarian cancer in both studies, and the risk estimate for the pooled studies was 1.36 (95% CI 1.04-1.77, P=0.03). There was also a suggestion that this risk may be greater for ovarian cancers of the serous subtype for both studies, with an OR (95% CI) of 1.66 (1.17-2.54) for the two studies combined (P=0.005). The *BRCA2* 372 *HH* genotype appears to be associated with an increased risk of ovarian cancer of a similar magnitude to that reported for breast cancer.

There was no evidence for a relationship between the variant allele and ovarian cancer risk for ***CYP19*** or ***HSD17B1*** [age adjusted OR (95%CI): *CYP19* 3'UTR heterozygous CT genotype 0.86 (0.60-1.24), *CYP19* 3'UTR homozygous TT genotype 0.70 (0.47-1.07); V allele of *HSD17B1* A238V 1.38 (0.35-5.49); heterozygous genotype of *HSD17B1* S313G 1.22 (0.87-1.72), homozygous GG genotype of *HSD17B1* S313G 0.98 (0.65-1.47)].

These odds ratios remained largely unchanged when tumours of low malignant potential (LMP) were excluded from the analysis. The genotyping protocol used for ***HSD17B1*** was used in the paper by Dunning et al (2004), on which Ms Livia Keleman (DoD-funded research assistant) is an author.

The +331G/A SNP in the progesterone receptor gene, ***PR***, was genotyped in a population-based, case-control study from North Carolina (438 cases and 504 controls) and in 535 cases and 298 controls from the Survey of Woman's Health (Berchuck et al., 2004). This SNP, which is in the *PR* promoter, alters the relative abundance of the A and B isoforms and has been associated with an increased risk of endometrial and breast cancer. There was a suggestion of a protective effect of the +331A allele (AA or GA) against ovarian cancer in the North Carolina study (OR = 0.72, 95% CI = 0.47–1.10). Examination of genotype frequencies by histologic type revealed that this was due to a decreased risk of endometrioid and clear cell cancers (OR = 0.30, 95% CI = 0.09-0.97). Similarly, in the Australian study there was a non-significant decrease in risk of ovarian cancer among those with the +331A allele (OR 0.83, 95% CI = 0.51–1.35) that was strongest in the endometrioid/clear cell group (OR 0.60, 95% CI = 0.24–1.44). In the combined US/Australian data that included 174 endometrioid/clear cell cases (166 invasive, 8 borderline) the +331A allele was significantly associated with protection against this subset of ovarian cancers (OR 0.46, 95% CI = 0.23–0.92). Preliminary evidence of a protective effect of the +331A allele against endometriosis also was noted in control subjects (OR = 0.19, 95% CI = 0.03-1.38). These findings suggest that the +331G/A progesterone receptor promoter polymorphism may modify the molecular epidemiologic pathway that encompasses both the development of endometriosis and its subsequent transformation into endometrioid or clear cell ovarian cancer.

We found that there was a trend for increased ovarian cancer risk associated with the L allele of the ***SRD5A2*** V89L polymorphism (age adj OR=1.30; 95% CI=1.03-1.62; p_{trend}=0.03) which was also apparent among the invasive tumours only (OR=1.25; 95% CI=0.98-1.59; p_{trend}=0.08). A significant trend was observed for reduced ovarian cancer risk associated with the R allele of the ***HSD17B4*** W511R polymorphism (age adj OR=0.68; 95% CI=0.47-0.97; p_{trend}=0.04); the trend remained similar for the invasive tumours (age adj OR=0.75; 95% CI=0.51-1.09; p_{trend}=0.14). Genotype frequency differences across ovarian cancer subgroups defined by tumour characteristics (including histology) are being explored.

Task 3 To genotype the cases and controls from the Australian Ovarian Cancer Study for 16 SNPs (months 25-42)

DNA has been extracted from a total of ~1730 cases and controls. Of these, ~1704 samples have been quantitated and diluted before plating 384 well plates. In addition, ~150 control and ~450 case blood pellets await extraction. A further 245 case samples and 317 control samples from a related project, the Australian Cancer Study (ACS; PI - Professor Adele Green) have also been quantitated and plated into 384 well plates. Genotyping of the AOCS and ACS cases and controls has commenced with genotyping of following SNPs:

Progesterone Receptor (*PR*)	G331A
Aromatase (*CYP19*)	C>T 3'UTR
5alpha-reductase (*SRD5A2*)	Val89Leu

17βhydroxysteroid dehydrogenase (*HSD17B1*)	Ala238Val
17βhydroxysteroid dehydrogenase (*HSD17B1*)	Ser313Gly
17βhydroxysteroid dehydrogenase (*HSD17B4*)	Trp511Arg
BRCA2	Asn372His
X-ray cross complementation (*XRCC2*)	Arg188His
X-ray cross complementation (*XRCC3*)	Thr241Met
RAD52	Tyr418Ter

Task 4 To perform genotyping for 2 short tandem repeat (STR) polymorphisms on both case-control studies (months 36-42)

Not started because DNA extraction is ongoing (see above).

Task 5 Statistical analysis of the genotyping results from the Survey of Women's Health Study and controls from the Australian Breast Cancer Family Study (months 24-36)

Analysis of the SWH and ABCFS control data has been completed, and there was preliminary evidence from the SWH that four of them (in *PR*, *BRCA2, SRD5A2* and *HSD17B4*) are associated with ovarian cancer risk.

Task 6 Full statistical analysis of the genotyping results (months 40-48) and preparation of manuscripts

We are therefore analysing the data from ACOS/ACS to try to validate these findings and expect to submit a manuscript in the next three months.

PUBLICATIONS

Auranen, A., Spurdle, A.B., Chen, X., Lipscombe**,** J., Purdie, D.M., Hopper, J.L., Green, A., Healey, C.S., Redman, K., Dunning, A. M., Pharoah, P. D., Easton, D., Ponder, B.A.J., **Chenevix-Trench, G**., and Novik, K.L. BRCA2 Asn372His polymorphism and epithelial ovarian cancer risk. *International Journal of Cancer* 103:427-30 (2003)

Dunning AM, Dowsett M, Healey CS, Tee L, Luben RN, Folkerd E, Novik KL, Kelemen L, Ogata S, Pharoah PD, Easton DF, Day NE, Ponder BA. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst. 96(12):936-45 (2004)*

Berchuck, A., Shildkraut, J., Wenham, R.M., Calingaert, B., Ali, S., Henriott, A., Halabi, S., Rodgriguez, G.C., **Gertig, D**., Purdie, D,M., Keleman, L., Spurdle, A.B., Marks, J. and **Chenevix-Trench**, G. Progesterone receptor +331A polymorphism is associated with a reduced risk of endometrioid and clear cell ovarian cancers. *Cancer Epidemiology, Biomarkers and Prevention* 13:2141-7 (2004)

Webb, P.M., Hopper, J.L., Newman, B., Chen, X., Keleman, L., McCredie, M.R.E., Southey, M. Giles, G.G., **Chenevix-Trench,** G. and Spurdle, A.B. Double strand break repair gene

polymorphisms and risk of breast or ovarian cancer. *Cancer Epidemiology, Biomarkers and Prevention* 14:319-23 (2005)

Kelemen, L., Spurdle, A.B., Purdie, D.M., **Gertig, D**. and **Chenevix-Trench**, G. *RAD52* Y415X truncation polymorphism and epithelial ovarian cancer risk in Australian women. *Cancer Letters* 218:191-7 (2005)

Tinker AV, Boussioutas A, **Bowtell DD**. The challenges of gene expression microarrays for the study of human cancer. Cancer Cell. 2006 May; 9(5):333-9. Review.

KEY RESEARCH ACCOMPLISHMENTS

Cores A and B

We have met our targets for patient recruitment and data/sample collection (See Reports for Core A Epidemiology and Core B Biospecimens). In relation to this we also have established systems to manage data and samples from all of the different sites.

Project 1

Gene expression markers of potential prognostic significance have been identified and these need to validated on additional datasets. We have identified a set of genes with robust expression differences between LMP and invasive tumours. We have also developed a classification tool for Krukenberg and Krukenberg-like tumours, which is likely to be of considerable value for determining the origin of atypical ovarian tumours, especially mucinous ovarian tumours.

Project 2

We have conducted the first case-control comparisons for serous cancers of the fallopian tube and peritoneum and have compared these to risk factors for ovarian cancer in the same study population. Our data suggest that primary serous ovarian and fallopian tube cancers share a common etiology while primary peritoneal cancers differ in several respects, this information has implications for our understanding of the process of ovarian carcinogenesis and we have recently submitted a manuscript for publication. Further data analysis is underway comparing risk factors for the different histological subtypes of ovarian cancer and for borderline and invasive tumours and we anticipate having several more papers submitted for publication by the end of 2006.

Project 3

The most recent novel finding from the samples from the Survey of Women's Health, in collaboration with Dr Andrew Berchuck, is that the +331A allele of the PR gene is significantly associated with protection against endometrioid ovarian cancers (OR 0.46, 95% CI = 0.23–0.92). This finding is currently the subject of a validation study by a large, new international consortium (the Ovarian Cancer Association Consortium) of 4500 cases and 7000 controls.

REPORTABLE OUTCOMES

Papers submitted for publication-

Jordan SJ, Green AC, Whiteman DC, Bain CJ, Gertig D, Webb PM, the Australian Ovarian Cancer Study Group and the Australian Cancer Study (ovarian cancer). Determinants of serous ovarian cancers: a new perspective. *Submitted to J Natl Cancer Inst, July 2006*.

Manuscripts in preparation-

Jordan SJ, Green AC, Whiteman DC, Webb PM, the Australian Ovarian Cancer Study Group and the Australian Cancer Study (ovarian cancer). A comparative analysis of risk factors for benign, borderline and invasive mucinous ovarian tumours: separate entities or a neoplastic continuum? *To be submitted for publication September 2006*.

Beesley J, Jordan SJ, Spurdle AB, Hopper JL, Webb PM, Chenevix-Trench, G, Australian Cancer Study (Ovarian Cancer), Australian Ovarian Cancer Study Group. SNPs in DNA repair and estrogen metabolism genes in the Survey of Women's Health and the Australian Ovarian Cancer Study. *To be submitted for publication September 2006*.

Jordan SJ, Green AC, Whiteman DC, Webb PM, the Australian Ovarian Cancer Study Group and the Australian Cancer Study (ovarian cancer). A comparative analysis of risk factors for benign, borderline and invasive serous ovarian tumours. *In preparation, to be submitted for publication December 2006*.

Olsen C, Webb PM, Whiteman DC, Green AC, the Australian Ovarian Cancer Study Group and the Australian Cancer Study (ovarian cancer). Obesity, physical activity and risk of ovarian cancer. *In preparation, to be submitted for publication December 2006*.

Nagle C, Webb PM, Whiteman DC, Green AC, the Australian Ovarian Cancer Study Group and the Australian Cancer Study (ovarian cancer). Risk factors for endometrioid ovarian cancer: a comparison to endometrial cancer. *In preparation, to be submitted for publication early 2007*.

Abstracts/Presentations
David Bowtell

2003 Helene Harris Memorial Trust 9th International Biennial Forum on Ovarian Cancer, Stratford-upon-Avon, United Kingdom
DOD (Department of Defense) Congressionally Directed Medical Research Programs, US Army Medical Research & Materiel Command, Fort Detrick, Frederick, USA
Familial Cancer 2003 – Research and Practice: KconFab, Australian Ovarian Cancer Study (AOCS) & Family Cancer Clinics of Australian and New Zealand, Couran Cove, Queensland

2004 Affymetrix Annual Partners Conference, Washington DC, USA
3rd International Consensus Workshop on Ovarian Cancer in Beuhl/Baden-Baden Germany
Scottish Gynaecological Cancer Trials Group, Glasgow, Scotland
Hutchinson/MRC Research Centre, Addenbrooke's Hospital, University of Cambridge, United Kingdom

2005 Current Perspectives and New Horizons in Ovarian Cancer, Melbourne
New Zealand Society for Oncology Conference 2005 "Stepping forward" Te Papa, Wellington
kConFab, Australian Ovarian Cancer Study (AOCS), The Family Cancer Clinics of Australia and the Australasian "Colorectal Cancer Family Study (ACCES) combined meeting, Queensland
2nd Affymetrix Asia-Pacific User Group Meeting, Singapore

2006 kConFab, Australian Ovarian Cancer Study (AOCS) and the Family Cancer Clinics of Australia combined meeting, Queensland

Georgia Chenevix-Trench

2006 kConFab, Australian Ovarian Cancer Study (AOCS), and the Family Cancer Clinics of Australia combined meeting, Queensland

Penelope Webb

2006 Epidemiological insights into ovarian carcinogenesis – the first results from the Australian Ovarian Cancer Study. Australasian Epidemiology Association, Melbourne, Australia.

Risk factors for serous ovarian, primary peritoneal and fallopian tube cancer: epidemiological insights into ovarian carcinogenesis. kConFab, Australian Ovarian Cancer Study (AOCS), The Family Cancer Clinics of Australia and the Australasian "Colorectal Cancer Family Study (ACCES) combined meeting, Queensland, Australia.

Funding awarded based on work supported by this award

New South Wales Cancer Council & Queensland Cancer Fund. Quality of life and psychosocial predictors of outcome in a population based study of ovarian cancer. 2005-2007. AU$350,000.

Queensland Cancer Fund. Folate and related micronutrients, folate metabolising enzymes and risk of ovarian cancer. 2006-2007. AU$150,000.

Australian Cancer Councils- Molecular Epidemilogy Of Ovarian Cancer: Australian Ovarian Cancer Study- Western Australia, Tasmania And A National Clinical Follow-Up Core, 2003-2007 AU$1,210,334

NHMRC Enabling Grant- Australian Ovarian Cancer Study (AOCS): A multidisciplinary ovarian cancer resource for the genomics era, 2006-2010. AU$1,000,000

NHMRC Project Grant- Molecular Epidemiology of Ovarian Cancer: The Australian Ovarian Cancer Study National Clinical Follow-Up Core, 2006-2010. AU$830,000

NHMRC Project Grant - Microarray-targeted candidate gene approach to finding ovarian cancer susceptibility genes, 2006-2008 AU$601,250

Department of Defence - Identification of ovarian cancer susceptibility genes involved in stromal-epithelial cross talk, 2006-2008 AU$645,625

CONCLUSIONS

Cores A and B

Recruitment of cases for the study began in January 2003 and control recruitment began in May 2003. Full population-based recruitment of cases ceased for women diagnosed after 30 June 2005 but collection of biospecimens and limited epidemiological data continued at key centres until June 300 2006. We have recruited a total of 1707 women with ovarian cancer (with an additional 296 women recruited since 1 July 2005 for the biospecimens extension) and 1073 control women. In addition, we have collected 1719 questionnaires, 1694 blood samples and 1061 frozen tissue samples. The recruitment, sample and data collection and processing systems have worked well and we have exceeded our targets as outlined in the reports from Core Components A (Epidemiology) and B (Biospecimens).

Project 1
Initial array analysis on archival samples has been in progress throughout 2006 leading to the discovery of several marker genes. We have switched further microarray expression analysis of ovarian cancer samples to an Affymetrix U1332.0+ array system. The intention is to array 500 samples, including the previously tested archival samples and the, as yet untested, prospective samples on U1332.0+ arrays, thereby creating a database of 100's of well-defined ovarian samples. To date, over 400 AOCS cases have been selected (based on amount of clinical information available) and randomised. A total of 310 cases have been arrayed and initial GeneSpring analysis has commenced. We expect publications by the end of 2006.

Project 2
We have conducted the first case-control comparisons for serous cancers of the fallopian tube and peritoneum and have compared these to risk factors for ovarian cancer in the same study population. Our data suggest that primary serous ovarian and fallopian tube cancers share a common etiology while primary peritoneal cancers differ in several respects, this information has implications for our understanding of the process of ovarian carcinogenesis and we have recently submitted a manuscript for publication. Further data analysis is underway comparing risk factors for the different histological subtypes of ovarian cancer and for borderline and invasive tumours and we anticipate having several more papers submitted for publication by the end of 2006. Future analyses will link the epidemiology data from project 2 with genetic data from project 3 and molecular data from project 1.

Project 3
Of the eleven polymorphisms nominated for analysis in this project, there is preliminary evidence from the SWH that four of them (in *PR*, *BRCA2, SRD5A2* and *HSD17B4*) are associated with ovarian cancer risk. Further analyses in the AOCS samples will provide independent testing of these SNPs in ovarian cancer risk, and if confirmed provide more power to look for associations with subtypes of ovarian cancer, and to start to look for gene-gene and gene-environment interactions

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APPENDICES

None